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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY OF SEVERAL PRESERVATIVES TO MICROORGANISMS

W. V. CRUESS,[1] P. H. RICHERT,[2] AND J. H. IRISH[3]

In the preservation of food products commercially by means of sodium benzoate or of sulfurous acid, marked irregularities in pre servative effect have been observed. In experiments conducted in this laboratory several years ago at the request of the industries concerned, it was found that 1/10 of 1 per cent sodium benzoate failed to prevent the spoiling of ripe olives, artichokes, avocado pulp, and sliced avocadoes in brine, whereas more acid products, such as fruit juices and green olives, were preserved satisfactorily by this concentration of benzoate. In other experiments it was found that potassium metabisulfite ($K_2S_2O_5$, the anhydride of $KHSO_3$) was a much less effective preservative for juice from overripe grapes than for juice from slightly immature grapes. It appeared, therefore, that sodium benzoate and potassium metabisulfite are more effective as preservatives in media of high acidity than in those of low acidity.

Because of these and other observations, it was suspected that the reaction of the medium, that is, its hydrogen-ion concentration, probably plays an important rôle in the preservation of food products by sodium benzoate and sulfurous acid (or its salts).

A study of papers on the toxicity of various reagents to microorganisms showed that previous attention had been given principally to their disinfecting power, that is, killing action, rather than to their

[1] Associate Professor of Fruit Products and Chemist in the Experiment Station.

[2] Research Assistant in Fruit Products, resigned July 1930.

[3] Junior Chemist in the Experiment Station.

preservative effect. However, Herter (1910) reported that 0.2 per cent of sodium benzoate retarded growth of and gas production by *Bacillus coli* in plain glucose broth, but had no noticeable effect in the same medium in the presence of calcium carbonate. He made no determination of hydrogen-ion concentration and gave the factor of acidity only passing attention. Barnard (1911) stated that benzoic acid is a more effective preservative than sodium benzoate but cited no experimental evidence to support his statement.

Held (1915) found that benzoic acid was more effective in a medium of low than in one of high protein content. He stated that when the protein was 'bound' by some other acid, such as tartaric, the concentration of sodium benzoate necessary for disinfection was lessened.

Perry and Beal (1920) found that 0.5 per cent of sodium benzoate was required to prevent the growth of the yeast *Saccharomyces cerevisiae* and 3.0 per cent was required to kill the cells. They also stated that benzoic acid was more effective than sodium benzoate in preventing growth of yeasts and molds.

Bonacorsi (1923) reported that the pH value of the medium greatly affected the killing action of several common disinfectants on microorganisms.

Fleischer and Amster (1922) reported that the disinfecting action of acid dyes was enhanced by a decrease in pH value and that of basic dyes by an increase in pH value.

Waterman and Kniper (1925) stated that the inhibitive action of the salts of cinnamic, salicylic, and benzoic acids on *Penicillium glaucum* was much lower than that of the free acids.

Kuroda (1926) found that the disinfecting action of several phenols and aromatic acids on *Bacillus coli* and *B. prodigiosus* was much greater at pH values of 1.4 to 3.5 than at pH 5.0 to 8.9.

Cruess and Richert (1929) published a preliminary report of certain observations made upon the effect of pH value on the inhibiting action of sodium benzoate on the growth of several common food spoilage microorganisms; the inhibiting action was much greater at pH values below 4.5 than at 4.5 to 8.0.

Behre (1930) has summarized previously existing information in a recent article on food preservatives, including relation of pH value to preservative action.

The data obtained in our investigations are presented separately for each preservative and in the following order: sodium benzoate, sodium salicylate, sodium sulfite ('sulfurous acid'), 'acetic acid,' sodium chloride, and formaldehyde. These experiments were con-

ducted with pure cultures. However, in connection with this investigation tests of a practical nature were also made with mixed cultures and commercial food products such as olives, asparagus, avocado pulp, etc., in order to determine whether the results with pure cultures were applicable in commercial practice. The results of these experiments appeared in a technical journal article by Cruess (1931).

SODIUM BENZOATE

The microorganisms used with sodium benzoate were *Saccharomyces ellipsoideus* (strain 66) isolated from naturally fermenting California grape juice, one strain of *Mycoderma* isolated from cucumber pickle brine and another isolated from fermented apple juice, two species of *Penicillium*, a gray *Mucor* isolated from fresh fruit, a culture of lactic bacteria (E. B. Fred's culture No. 124–2), vinegar bacteria culture from cider vinegar, and cultures of *Bacillus coli*, *B. sporogenes*, *B. subtilis*, and *B. botulinus* from the Bacteriology Department of the University of California.

Concentrations of Sodium Benzoate to Prevent Growth in Liquid Media at Various pH Values.—For the vinegar bacteria and lactic acid bacteria, grape juice and apple juice were used; for *Bacillus coli*, *B. subtilis*, and *B. sporogenes*, a broth of the following composition was prepared: bacto-pepton, 10.0 grams; Libby's extract of beef, 10.0 grams; glucose, 7.5 grams; $MgSO_4$, 0.01 gram; KH_2PO_4, 0.25 gram; $(NH_4)_2\ HPO_4$, 0.25 gram; and water to make 1,000 cc. The three media were brought to various pH values by the addition of citric acid or sodium hydroxide. They were then subdivided into 100 cc portions to which were added amounts of benzoate ranging from none to an amount at each pH value which preliminary tests indicated to be sufficient to prevent growth. The liquids were then placed in plugged tubes and sterilized at the temperature and time required for the pH value concerned (as determined by preliminary trials); thus, liquids below pH 4.5 were heated one hour at 100° C, and those above pH 5.0, were sterilized by the usual intermittent three-period heating at 100° C. Since the pH values in some cases changed considerably during sterilization, the values reported in the tables are those taken after sterilization.

The buffering effect of the sodium benzoate is evident. In alkaline solutions the decrease in pH value is probably due to the formation of organic acids by the action of the alkali on the hexose sugars. The changes in pH value of liquids of high and of low pH value in the presence of the benzoate were, in most cases, greater than those in its absence. It tended to buffer to a pH value of 5.4–5.5.

TABLE 1

TYPICAL CHANGES IN pH VALUE DURING STERILIZATION OF CULTURE MEDIA

No benzoate added		Benzoate added in amounts shown		
pH value before sterilization	pH value after sterilization	Per cent benzoate added	pH value before sterilization	pH value after sterilization
3.2	3.6	0.05	3.2	3.6
4.0	3.9	0.15	4.0	4.0
4.4	4.5	0.15	4.4	4.6
5.6	5.0	1.50	5.6	5.4
6.0	5.7	1.50	6.0	5.5
7.4	7.3	1.50	7.4	6.1
9.0	8.6	1.50	9.0	7.4
10.0	8.7	1.50	10.0	7.9

Transfers of the various pure cultures, previously listed, were made to the sterilized tubes of media representing the various pH values and benzoate concentrations. The fermentation organisms used for inoculation were grown for five days in grape juice; the molds were grown on grape juice until abundant formation of spores had occurred; and the acid-intolerant organisms were grown in nutrient broth at 37° C for 5 days before transfer to the tubes of media containing benzoate. In all cases noninoculated tubes were retained for comparison with the inoculated ones in order to facilitate detection of growth. The tubes were stored at room temperature for six months. Regular observations were made to determine evidence of growth. In most positive tubes growth made its appearance in less than two weeks. All cultures in which growth was doubtful were examined microscopically.

The effect of pH value on the concentration of benzoate to prevent growth may be seen from the data presented in tables 1 and 2. Owing to the fact that not all of the experiments were made simultaneously nor with media of the same range of pH values, the pH values are given in the tables for each organism.

Similar experiments were conducted with *Bacillus botulinus* (*clostridium botulinum*). An asparagus juice medium was prepared, tubed, stratified with neutral oil, and sterilized. Tubes in duplicate of the sterile media of several pH values ranging from pH 4.0 to 8.6 were inoculated with spores of *B. botulinus* (*cl. botulinum*) grown in brain medium and detoxified by heat. The culture from which the spores were taken was rapidly fatal to guinea pigs before detoxification and nontoxic to them after heating to detoxify. The spores were still active,

TABLE 2

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM BENZOATE NECESSARY TO PREVENT THE GROWTH OF ACID-TOLERANT MICROORGANISMS

Saccharomyces ellipsoideus from grapes		*Mycoderma* from cider		*Mycoderma* from pickle brine		*Penicillium* (green)	
pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth
2.3	0.02	2.5	0.03	2.7	0.05	2.5	0.02
2.8	0.06	3.6	0.05	3.8	0.06	3.6	0.03
3.5	0.09	4.3	0.08	4.7	0.50	4.3	0.08
4.0	0.10	5.0	0.25	5.4	1.00	5.0	0.30
4.9	0.40	6.5	More than 1.5	7.3	4.00	6.5	1.50
5.2	0.45	.....	.....	.....	.....	7.3	2.60
6.2	More than 1.5	.....	.....	10.0	0.70	8.5	More than 1.5
7.3	3.4	.....	.....	.....	.....	10.2	More than 1.5
9.5	More than 1.5	.....	.....	.....	.....	11.0	0.90

Penicillium (gray)		*Mucor*		Vinegar bacteria		Lactic bacteria	
pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth	pH value	Benzoate, grams per 100 cc to prevent growth
2.4	0.03	2.4	0.04	2.4	0.04	3.6	0.05
3.0	0.06	3.0	0.06	3.0	0.06	3.9	0.06
4.2	0.08	4.2	0.08	4.2	0.10	4.0	0.06
4.5	0.20	4.5	0.20	4.5	0.20	4.5	0.12
5.2	0.50	5.2	0.50	5.2	0.70	5.0	0.20
6.0	More than 1.5	6.0	1.20	6.0	More than 1.5	5.7	0.60
7.0	More than 1.5	.....	.....	7.0	More than 1.5	7.3	3.4
.....	.....	7.3	3.4	7.3	3.4	8.6	More than 1.5

TABLE 3

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM BENZOATE TO PREVENT GROWTH OF SEVERAL ACID-INTOLERANT MICROORGANISMS

pH value	*Bacillus coli*	*Bacillus subtilis*	*Bacillus sporogenes*
	Benzoate grams per 100 cc to prevent growth		
4.0	0.00	0.00	0.00
4.5	0.06	0.00	0.04
5.0	0.12	0.08	0.12
5.7	0.60	0.40	0.80
7.3	2.40	1.20	2.60
8.6	Growth at 1.5	Growth at 1.5	Growth at 1.5

however, after detoxifying by heat, for transfers from the detoxified suspension grew readily and produced toxin in brain medium.

At pH 8.6, 3.0 grams of sodium benzoate per 100 cc was required to prevent growth; at pH 7.4, 2.0 grams per 100 cc; at pH 5.2, 0.2 gram per 100 cc; at pH 4.7, 0.075 gram per 100 cc, and at pH 4.0, growth failed to occur even in the complete absence of benzoate.

At pH 7.4 with 0.8 gram of sodium benzoate per 100 cc the medium became fatally toxic to guinea pigs fed by mouth, whereas at pH 4.7 with 0.1 gram of benzoate per 100 cc the medium remained nontoxic and apparently free of growth. Owing to lack of facilities, the liquids in the other tubes were not tested in this manner. Growth was judged as positive or negative in these other tubes by macroscopical appearance, odor, and microscopical appearance of stained specimens.

Considering first the acid-tolerant organisms, it is evident that at pH values below 4.0 the benzoate exerts its greatest toxicity. At these values, in most instances, less than 0.1 gram of benzoate per 100 cc was required to prevent growth. Between pH 4 and pH 6 the concentration required to prevent growth increased rapidly, that is, the toxicity of the benzoate rapidly decreased over this pH range. The critical point appears to be in the neighborhood of pH 4.5, as there was a very sharp increase in the benzoate required to prevent growth when the pH value was increased from 4.5 to 5.0 or 5.2. From pH 5.0 to 7.3 the increase was less rapid, and tolerance appeared to approach a maximum between pH 7.3 and 10.0. The fact that less benzoate was required to prevent growth of the *Mycoderma* and *Penicillium* at pH 10.0 than at pH 7.3 would indicate that the maximum tolerance of these organisms for benzoate lies between these two pH values and that beyond this maximum the concentration required to prevent growth decreases. *Saccharomyces ellipsoideus* failed to grow at pH 10.0, even in the absence of benzoate.

The data clearly show that the toxicity of sodium benzoate to the yeasts, molds, vinegar bacteria, and lactic bacteria used in these experiments is greatly affected by the pH value of the medium.

Of the organisms studied, the *Mycoderma* appeared to be the most resistant to the benzoate at pH values on the acid side of neutrality.

Considering next the organisms intolerant of acid in table 3, it will be observed that they also were much less resistant to sodium benzoate at pH values of 5.0 or less than at pH values of 5.7 to 8.3. None of these organisms grew at pH values of 4.0 or less, even in the absence of benzoic acid.

Bacillus subtilis proved less resistant than *B. coli* and *B. sporogenes* to sodium benzoate, and all three cultures were less resistant than the

acid-tolerant organisms of table 2. However, it is possible that at pH 7.3 the relatively high concentration of sodium ion resulting from neutralization of the medium and from the added benzoate exerted a slight toxic effect separate from that of the benzoate ion (granting that the benzoate ion is toxic, a doubtful assumption). The evidence indicates that the undissociated benzoic acid is the toxic agent.

Considering the data of the two tables as a whole, the evidence is conclusive that the pH value very greatly affected the concentration of sodium benzoate to prevent growth. In order to give this statement greater emphasis, the average concentrations of sodium benzoate to prevent growth at several pH values have been calculated, making use of the data given for the organisms of table 2. (See table 4 and fig. 1.)

TABLE 4

AVERAGE VALUES OF SODIUM BENZOATE TO PREVENT GROWTH OF ACID-TOLERANT MICROORGANISMS AT SEVERAL pH VALUES

pH value	Benzoate, grams per 100 cc to prevent growth	Number of species of microorganisms represented
2.3–2.5	0.030	6
2.7–3.0	0.060	4
3.5–4.3	0.082	7
4.5–4.7	0.245	5
4.9–5.2	0.407	7
5.7–6.0	1.100	3
7.3–10.0	3.370	6
10.0–11.0	0.800	2

Only two cultures grew at pH values of 10.0–11.0, and it is of interest to note that the data indicate that less benzoate is required to prevent growth of these two organisms (a *Mycoderma* and a mold) than at or near neutrality. Evidently the OH ion reduces their vigor.

Relation of pH Value to the Inhibiting Action of Sodium Benzoate on the Multiplication of Yeast.—In experiments similar to those presented in table 5, it was noted that growth of various microorganisms was less abundant, for a given pH value, at high than at low concentrations of sodium benzoate. Apparently, also, the retarding effect was relatively less pronounced at pH values near neutrality than at those at or below pH 4.

In order to obtain an approximate numerical measure of the observed retarding effect of sodium benzoate, sterilized 100 cc portions of filtered apple juice of pH 3.0, 3.9, and 6.0 containing the concentrations of benzoate given in table 5 were inoculated with approximately 1,200,000 cells of *Saccharomyces ellipsoideus* per 100 cc. At intervals the number of cells per cc in each sample was determined

by counting under the dry high power of a microscope equipped with a calibrated net eye piece. The samples taken for counting were diluted to a definite volume with water and mounted in a hemocytometer. Three mounts were made of each specimen and the cells in 25 or more squares were counted for each mount.

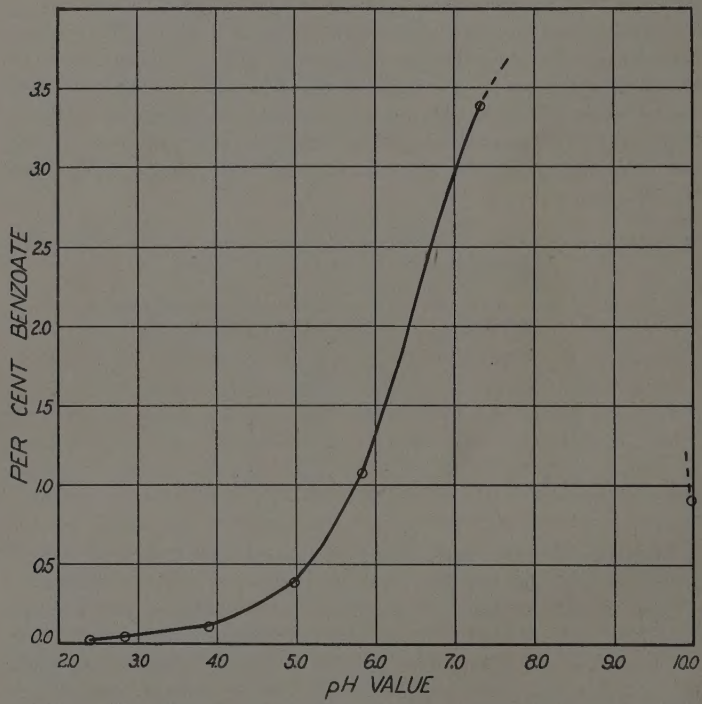

Fig. 1. The effect of pH value on sodium benzoate to prevent growth. Average for several acid-tolerant microorganisms. The solid line shows averages of data; the dotted line, the hypothetical curve from pH 7.3 to 10.0. The point for pH 10.0 is based on data for two organisms only, since most organisms failed to grow at this pH value, even in the absence of preservative.

The data show that the benzoate exerted a definite retarding effect on the multiplication of *Saccharomyces ellipsoideus*, except at pH 6.0 with 0.01 gram benzoate per 100 cc; in this case the benzoate appeared to exert little or no effect. At pH values of 3.0 and 3.9, however, 0.01 gram of benzoate per 100 cc greatly retarded multiplication. A concentration of 0.05 gram of benzoate per 100 cc permitted an increase in 6 days to 58,000,000 cells per cc at pH 6.0; an increase to only

4,800,000 at pH 3.9 and no increase whatsoever at pH 3.0. While counting bacterial cells by microscope is a difficult and rather inaccurate procedure, counting yeast cells in this manner gives reasonably consistent results. The differences reported in table 5 between the numbers of cells in samples of different pH values, but of the same

TABLE 5

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM BENZOATE ON THE MULTIPLICATION OF SACCHAROMYCES ELLIPSOIDEUS

pH value	Benzoate, grams per 100 cc	Number of cells per cc at 6 days	Number of cells per cc at 14 days
3.0	0.00	58,000,000	93,000,000
3.0	0.01	19,000,000	56,000,000
3.0	0.05	No growth	No growth
3.9	0.00	60,000,000	106,000,000
3.9	0.01	36,000,000	67,000,000
3.9	0.05	4,800,000	41,000,000
3.9	0.10	No growth	No growth
6.0	0.00	67,000,000	108,000,000
6.0	0.01	70,000,000	103,000,000
6.0	0.05	58,000,000	86,000,000
6.0	0.10	19,500,000	78,000,000
6.0	0.20	9,600,000	75,000,000
6.0	0.30	2,400,000	33,000,000
6.0	0.40	1,900,000	4,000,000
6.0	0.60	No growth	No growth

benzoate concentrations, are so great that there is no doubt concerning their general significance. Thus, the number of cells at the end of 6 days at pH 3.0 and 0.01 gram benzoate per 100 cc was 19,000,000 per cc; whereas at pH 6.0 with 0.01 gram benzoate per 100 cc it was 70,000,000. It is also evident that the benzoate at certain concentrations retards, but does not completely inhibit, multiplication of *S. ellipsoideus;* compare pH 6.0 and 0.01 or 0 grams benzoate per 100 cc with pH 6.0 and 0.4 gram benzoate per 100 cc. It is interesting to observe from table 5 that there is evidence of some stimulating effect of the benzoate at pH 6.0 and 0.01 gram of benzoate per 100 cc. However, the evidence of such an effect is considerably stronger in table 7. See discussion of this point following table 7.

Retarding Action of Sodium Benzoate on the Rate of Alcoholic Fermentation at Various pH Values.—In one experiment, 100 cc portions of sterile apple juice of pH values of 3.0, 3.9, and 6.0 and containing the concentrations of sodium benzoate indicated in table 6 were each inoculated with 1 cc of a vigorously fermenting culture of *Saccharomyces ellipsoideus,* strain 66. After three months' storage at room temperature the Brix degree of each sample was determined by means of an accurate hydrometer graduated to 1/10° Brix. Decrease in

Brix degree was taken as an indication of an increase in the extent of fermentation. Fermentation at this time had ceased in all cultures.

At pH 3.0 fermentation was completely inhibited by 0.05 gram of benzoate per 100 cc whereas at pH 6.0 some fermentation and active growth occurred at 0.6 gram of benzoate per 100 cc. Apparently, some growth also occurred at 0.8 gram of benzoate per 100 cc and pH 6.0. The benzoate was at least 16 times as toxic to *Saccharomyces*

TABLE 6

EFFECT OF pH VALUE OF APPLE JUICE ON FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

pH value	Sodium benzoate, grams per 100 cc	Brix degree average of duplicates	pH value	Sodium benzoate, grams per 100 cc	Brix degree average of duplicates
3.0	0.00	0.4	6.0	0.00	0.5
3.0	0.01	3.7	6.0	0.01	0.5
3.0	0.05	15.5	6.0	0.05	0.5
3.9	0.00	0.5	6.0	0.10	0.6
3.9	0.01	0.7	6.0	0.20	10.5
3.9	0.05	11.0	6.0	0.30	14.0
3.9	0.10	15.4*	6.0	0.40	12.2
3.9	0.20	15.5	6.0	0.60	13.0
Sterile check	0.00	15.5	6.0	0.80	15.0

* A decrease of less than 0.5° Brix is probably not significant owing to possible variation in the loss of moisture by evaporation from the individual cultures during the long incubation period.

ellipsoideus at pH 3.0 as at pH 6.0, judged by its effect on fermentation.

The retarding action of sodium benzoate on the rate of fermentation was investigated further in several series of experiments by measuring the loss in weight of duplicate inoculated 100 cc samples of apple juice representing three pH values and several concentrations of sodium benzoate. The results from one such experiment are given in table 7, and several selected fermentation curves representing the three pH values are presented in figure 2.

It is seen from table 7 that a given concentration of sodium benzoate, for example, 0.02 gram per 100 cc, retarded the rate of fermentation much less at pH 4.5 than at pH 3.0. Likewise 0.1 gram of benzoate per 100 cc retarded fermentation considerably less at pH 7.0 than at 4.5. There was some stimulation of fermentation at pH 4.5 with 0.02 and 0.04 grams of benzoate per 100 cc and at pH 7.0 with 0.1 and 0.2 grams per 100 cc. This observation was in accord with the general principle that small concentrations of antiseptics stimulate the activity of microorganisms.

Four other series of fermentations were conducted with results similar to those reported in table 7. These definitely supported the

finding that at each pH value there exists a concentration below which the preservative stimulates and above which it retards yeast activity.

Growth of Penicillium Mold in 10 Per Cent Sodium Benzoate Solution.—In addition to the systematic experiments made to determine the relation of pH value to the toxicity of sodium benzoate to microorganisms, the following interesting chance observation was made:

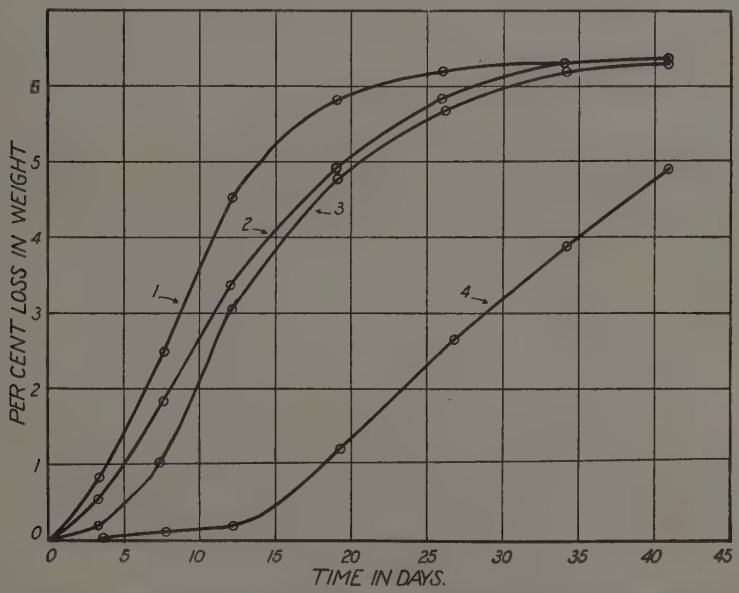

Fig. 2. Effect of pH value on the retarding action of sodium benzoate on yeast fermentation.

1. pH 4.5 and 0.0 grams per 100 cc
2. pH 7.0 and 0.2 gram per 100 cc
3. pH 4.5 and 0.06 gram per 100 cc
4. pH 3.0 and 0.02 gram per 100 cc

A liter bottle containing about 400 cc of 10 per cent sodium benzoate solution with a pH value of approximately 7.5, prepared in July, 1929, and stored at room temperature had developed a considerable quantity of cotton-like mycelial growth of a mold by November, 1930. By plating on nutrient agar agar the mold proved to be a strain of *Penicillium glaucum.* Spore formation was absent in the benzoate solution but was profuse on the agar plates.

This observation illustrates in a very striking manner the extremely low toxicity of sodium benzoate in solutions near neutrality.

Relation of pH Value and Benzoate to Preserve Several Food Products.—It was found that spoiling of cubed melon preserves, maraschino style grapes, ripe olives, avocado pulp, prune pulp, car-

TABLE 7

EFFECT OF pH VALUE ON THE RETARDING EFFECT OF SODIUM BENZOATE ON FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

Time in days	Benzoate, grams per 100 cc at pH 3.0		Benzoate, grams per 100 cc at pH 4.5						Benzoate, grams per 100 cc at pH 7.0							
	0	0.02	0	0.02	0.04	0.06	0.08	0.10	0	0.1	0.2	0.4	0.6	0.8	1.0	1.5
	Loss in weight in grams per 100 cc															
4	0.90	0.06	1.03	0.85	0.88	0.11	0.00	0.00	0.46	0.65	0.48	0.32	0.08	0.00	0.00	0.00
7	2.29	0.08	2.45	2.67	2.79	1.09	0.13	0.04	1.33	1.78	1.44	1.22	0.23	0.26	0.19	0.12
12	3.96	0.11	4.56	4.96	5.04	3.10	1.44	0.22	2.75	3.32	2.71	2.54	0.57	0.66	0.44	0.33
19	5.41	1.15	5.86	6.11	6.21	4.83	2.91	1.47	3.46	4.86	4.16	3.83	1.03	0.97	0.60	0.51
26	5.97	2.54	6.14	6.40	6.51	5.76	3.76	2.27	5.16	5.88	5.37	5.13	1.31	1.14	0.75	0.61
34	6.19	3.82	6.21	6.46	6.64	6.20	4.23	2.75	5.54	6.32	5.97	5.73	1.52	1.41	0.93	0.73
41	6.23	4.85	6.37	6.50	6.70	6.53	4.77	3.22	6.19	6.37	6.46	6.50	1.87	1.68	1.18	0.84
47	6.29	5.35	6.47	6.54	6.74	6.72	5.08	3.51	6.54	6.93	6.70	6.66	2.15	1.89	1.34	1.02
56	.....	5.83	6.52	.....	.....	.....	5.51	3.77	6.85	7.05	6.82	6.94	2.59	2.24	1.69	1.15
88	.....	6.74	.....	.....	.....	.....	6.83	4.98	7.31	.....	.....	.....	4.05	3.56	2.41	1.69

bonated beverages, asparagus, string beans, green peas, and artichokes could be prevented with 1/10 of 1 per cent or less of sodium benzoate when the pH value did not exceed 4.0. Near neutrality 2 per cent of sodium benzoate failed to prevent growth of molds, yeast, and bacteria. Therefore, the principle that the preservative action of sodium benzoate depends upon the pH value of the medium applies to foods as well as to the various culture media previously reported in this paper. Details of these experiments are given in a recent article by Cruess (1931).

OTHER PRESERVATIVES

The effect of pH value of the medium on the toxicity of sodium salicylate, sodium sulfite ('sulfurous acid'), 'acetic acid,' sodium chloride, and formaldehyde was studied in a manner similar to that already described for sodium benzoate. The first three preservatives were chosen because, like sodium benzoate, they represent weak acids or salts of such acids; sodium chloride was chosen because it represents the important class of neutral salts; and formaldehyde, because it represents a group of nondissociating organic compounds that should not be affected chemically by the pH values used in these tests. Formaldehyde, sodium salicylate, and salicylic acid are no longer permitted as food preservatives.

Sodium Salicylate.—Using the procedure outlined earlier in this report for sodium benzoate, the concentrations of C.P. sodium salicylate required to prevent growth of four different microorganisms were determined, with the results given in table 8.

TABLE 8

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM SALICYLATE REQUIRED TO PREVENT THE GROWTH OF MICROORGANISMS

pH value	*Saccharomyces ellipsoideus*	*Mucor* mold	*Penicillium* mold	Mixed culture of acetic bacteria
	Salicylate to prevent growth, grams per 100 cc			
2.5	0.02	0.02	0.04	0.02
3.5-3.8	0.06	0.15	0.10	0.08
7.0	3+	3+	3+	3+

Growth was positive in all cultures of pH 7.0 containing 3 grams of sodium salicylate per 100 cc.

As with sodium benzoate, a very much higher concentration (at least 150 times greater) of sodium salicylate was required to prevent the growth of common fermentation organisms at pH 7.0 than at pH 2.5. The toxicity of the salicylate at the three pH values used was similar to that previously observed for sodium benzoate.

Fermentation tests with cider containing sodium salicylate were conducted as previously described for sodium benzoate, with the results given in table 9. Data for several of the fermentation tests have been omitted to reduce the width of the table.

TABLE 9

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM SALICYLATE ON THE RATE OF FERMENTATION BY SACCHAROMYCES ELLIPSOIDEUS

Time in days	pH 2.5		pH 3.5		pH 7.0		
	Salicylate concentration		Salicylate concentration		Salicylate concentration		
	0 gram per 100 cc	0.02 gram per 100 cc	0 gram per 100 cc	0.04 gram per 100 cc	0 gram per 100 cc	0.08 gram per 100 cc	2.0 grams per 100 cc
	Loss in weight in grams per 100 cc						
1	0.13	0.00	0.38	0.00	0.00	0.00	0.00
2	1.40	0.00	1.70	0.00	0.75	0.82	1.20
3	2.58	0.00	2.96	0.00	2.05	2.14	2.98
4	3.58	0.00	4.06	0.73	3.12	3.29	4.11
6	5.40	0.43	5.80	2.20	5.21	5.40	5.64
10	7.01	0.56	7.30	3.66	6.85	6.71	7.04
13	7.92	0.85	7.34	4.11	7.22	7.02	7.56
16	8.25	1.34	7.95	4.84	7.86	7.53	8.26
21	8.78	1.69	8.36	5.65	8.19	7.82	8.57
24	9.06	2.08	8.42	6.45	8.50	8.11	8.88
32	9.59	2.97	9.40	7.85	9.25	8.83	9.60
34	9.69	3.15	9.60	8.14	9.40	8.98	9.77
36	9.98	3.81	10.08	8.90	9.85	9.41	10.19

At pH 7.0 none of the concentrations of sodium salicylate used appreciably affected the rate of fermentation. At pH 2.5 and 3.5 beginning of fermentation was delayed by small concentrations of salicylate and the rate of fermentation was retarded.

Sodium Sulfite ('Sulfurous Acid').—In a manner similar to that described for sodium salicylate the effect of pH value on the toxicity of sodium sulfite to four different kinds of microorganisms was determined. The term 'SO_2' or 'sulfurous acid' is commonly used in expressing the concentration of sulfites in foods but when so used should be placed in quotation marks, since several compounds of sulfur are involved. While Na_2SO_3, sodium sulfite, was used as the source of 'SO_2', it is probable that at different pH values the sulfur exists in several different forms such as H_2SO_3 (undissociated), and as HSO_3 and SO_3 ions. From our results, it would seem that it is not the SO_3 ion that exerts the toxic action but more likely the undissociated H_2SO_3, or the HSO_3 ion, or possibly the anhydride, SO_2.

Inoculation, incubation, and observations were conducted as previously described for sodium salicylate. Table 10 gives the data obtained.

TABLE 10

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM SULFITE, EXPRESSED AS 'SO_2', REQUIRED TO PREVENT GROWTH OF MICROORGANISMS

pH value	*Saccharomyces ellipsoideus*		*Mucor* mold		*Penicillium* mold		Mixed bacteria	
	Initial 'SO_2' concentration to prevent growth*							
	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc
2.5	200	0.02	200	0.02	300	0.03	100	0.01
3.5	800	0.08	600	0.06	600	0.06	300	0.03
7.0	Above 5000	0.50	Above 5000	0.50	Above 5000	0.50	1000	0.10

* Parts per million multiplied by 0.0001 gives per cent: thus 200 p.p.m.=0.02 per cent.

The pH value of the medium exerted a very marked effect on the toxicity of the preservative. Thus, at pH 7.0 growth of three organisms occurred abundantly in the presence of 5,000 p.p.m., that is, 5,000 milligrams of 'SO_2' per liter (0.5 per cent). The bacteria, however, were less resistant and 1,000 p.p.m. prevented growth, even at pH 7.0.

The incubation period was 105 days, but observations were taken also at 3, 10, 63, and 79 days.

The concentrations given indicate the amounts of 'SO_2' added in the form of Na_2SO_3. Unquestionably, some 'SO_2' disappeared from the acidified samples as SO_2 gas and some of the SO_2 in all samples probably was oxidized to SO_3. Nevertheless, the results are qualitatively comparative and indicate that the preservative action of SO_2 is dependent upon the pH value of the medium.

Analyses of samples of pH 3.5 before and after sterilization gave the following results:

TABLE 11

LOSS OF 'SO_2' DURING STERILIZATION OF FRUIT JUICE

'SO_2' added as sulfite		'SO_2' after sterilization	
Parts per million	Grams per 100 cc	Parts per million	Grams per 100 cc
500	0.050	275	0.027
600	0.060	395	0.040
800	0.080	492	0.049
1000	0.100	672	0.067

The effect of pH value on the retarding action of 'SO_2' on fermentation by *Saccharomyces ellipsoideus* is shown in table 12.

TABLE 12

EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM SULFITE, EXPRESSED AS 'SO_2' ON YEAST FERMENTATION

Time in days	pH 3.5				pH 7.0				
	'SO_2', parts per million				'SO_2', parts per million				
	0	100	200	300	0	100	300	1,000	5,000
	Loss in weight in grams per 100 cc								
1	1.2	1.2	0.0	0	0.6	0.6	1.2	1.0	0.0
2	6.6	2.4	0.0	0	6.6	6.0	6.8	5.4	1.8
3	9.0	6.0	0.0	0	12.0	1.2	11.4	11.4	2.4
6	13.8	13.2	1.8	0	13.2	13.2	13.2	13.2	9.6
8	15.6	15.6	10.8	0	15.0	15.0	15.0	15.0	12.0

As was true of the other weak acids the retarding action of 'SO_2' on fermentation was greater at the relatively low pH value of 3.5 than at neutrality, pH 7.0.

Potassium Acetate and 'Acetic Acid'.—Potassium acetate was used as a source of acetate radical and citric acid or postassium hydroxide to give the desired pH values. Sodium acetate proved unsuitable because of the toxicity of the Na ion at the higher concentrations of acetate. The growth of *Saccharomyces ellipsoideus*, *Penicillium* mold, *Mucor* mold and *S. cerevisiae* was prevented at pH 3.5 by 0.8 to 1.0 grams of *'acetic acid'* per 100 cc, whereas at pH 7.0, 4.0 grams of 'acetic acid' (that is, potassium acetate to give a concentration of acetate radical equal to that of 4.0 grams of acetic acid in 100 cc) failed to prevent growth. At pH 3.5 vinegar bacteria developed at all concentrations of 'acetic acid' used. This is not surprising, since *Bacterium aceti* converts ethyl alcohol to acetic acid and in commercial practice produces vinegars containing 10.0 grams of acetic acid per 100 cc. As with 'SO_2', evidently the undissociated acid, CH_3CO_2H and not the CH_3CO_2 ion is the toxic agent.

The effect of pH value on the retarding action of potassium acetate expressed as 'acetic acid' on fermentation is indicated in table 13.

At pH 7.0 the maximum concentration (4.0 grams per 100 cc) of 'acetic acid' failed to retard fermentation noticeably, whereas at pH 3.5 the rate was markedly retarded at 0.3 gram per 100 cc and failed to occur within the duration of the experiment at 0.6 gram per 100 cc. Evidently the undissociated HAc is the toxic agent, as at pH 3.5

TABLE 13

EFFECT OF pH VALUE ON THE RETARDING ACTION OF POTASSIUM ACETATE, EXPRESSED AS 'ACETIC ACID,' ON THE RATE OF FERMENTATION

Time in days	'Acetic acid,' concentration in per cent at pH 3.5		'Acetic acid,' concentration in per cent at pH 7.0				
	0	0.3	0	0.3	0.6	1.0	1.5
	Loss in weight in grams per 100 cc						
1	1.0	0.0	2.5	1.5	1.5	1.0	0.0
2	8.0	0.0	8.0	8.0	8.0	5.0	2.5
3	11.0	3.0	11.0	11.5	10.5	8.5	4.0
4	13.0	8.5	12.5	12.5	12.5	11.5	9.0
5	14.0	10.0	13.5	13.5	14.0	13.0	12.5
7	14.5	12.0	14.5	14.5	14.5	13.5	13.5
9	15.5	13.0	15.5	15.0	15.0	14.5	14.5

most of the acid is in this form and at pH 7.0 the acetate used is practically completely dissociated into Na and CH_3CO_2 ions. The H ion is naturally involved, but it alone at pH 3.5 does not prevent yeast growth, for fruit juices of pH 2.5 containing 10 times the H ion concentration of those of pH 3.5 were fermented readily in other experiments reported in this paper (citric acid being used to furnish the H ions). See for example table 15.

Sodium Chloride.—The concentrations of sodium chloride to prevent growth of two molds and a yeast were affected in some degree by the pH value of the medium as shown by the results given in table 14.

TABLE 14

EFFECT OF pH VALUE ON THE CONCENTRATION OF SODIUM CHLORIDE TO PREVENT GROWTH OF MICROORGANISMS

pH value	*S. ellipsoideus*	*Mucor* mold	*Penicillium* mold
	Approximate concentration of sodium chloride in per cent, to prevent growth		
2.5	14	16	18
3.5	20	20	20
7.0	20	20	20

The incubation period was approximately three months at room temperature. Apparently, the pH value of the medium is of much less consequence in the preservation of foods with sodium chloride than with the preservatives discussed previously in this paper. The

results agree well with those reported by Joslyn and Cruess (1929) in similar experiments with *Mycoderma* yeasts. The effect of pH value on the retarding action of sodium chloride on the rate of fermentation is given in table 15.

TABLE 15

THE EFFECT OF pH VALUE ON THE RETARDING ACTION OF SODIUM CHLORIDE ON FERMENTATION

Time in days	Per cent sodium chloride at pH 2.5			Per cent sodium chloride at pH 3.5			Per cent sodium chloride at pH 7.0		
	0	3	6	0	3	6	0	6	9
	Loss in weight in grams per 100 cc								
2	1.85	1.11	0.69	1.96	1.67	0.69	1.98	0.88	1.11
4	3.87	1.29	0.87	4.62	3.25	1.17	3.95	1.03	1.29
6	5.76	1.62	1.15	6.93	4.26	1.47	5.74	1.57	1.50
9	8.02	2.27	1.59	8.45	7.22	2.12	8.07	1.70	.....
11	9.17	2.92	2.07	9.22	8.75	3.28	9.01	2.25	.....
18	11.75	3.75	2.33	10.16	10.30	4.24	9.79	4.51	2.61
23	12.24	4.97	3.64	11.84	11.99	5.58	11.34	7.65	3.24
26	13.46	6.17	4.59	13.04	13.48	7.42	12.53	9.26	4.31
30	14.30	7.10	5.26	13.93	14.31	8 74	13.52	10.58	5.29

The sodium chloride retarded growth somewhat more at pH 2.5 and 3.5 than at 7.0. Citric acid was used to decrease the pH value to 2.5 and sodium hydroxide to increase it to 7.0; that neither of these substances exerted any significant retarding action can be seen by comparing the data in the three columns entitled "0 per cent sodium chloride."

Formaldehyde.—The effect of pH value on the concentration of formaldehyde to prevent growth of the four organisms was slight. The results of the experiment are given briefly as follows: growth at pH 2.5 was prevented by 0.015 gram formaldehyde per 100 cc but not by 0.0075 gram; growth at pH 3.5 and 7.0 was prevented by 0.03 gram formaldehyde per 100 cc but not by 0.015 gram. Therefore, at a very high acidity the formaldehyde is somewhat more toxic to microorganisms than at moderate acidity, or at neutrality. The effect of pH value, however, is very much less than that found for sodium benzoate, sodium salicylate, and potassium acetate, and resembles that obtained with sodium chloride.

Using the technique previously described, the effect of pH value on the retarding action of formaldehyde on fermentation was studied with the results given in table 16.

TABLE 16

EFFECT OF pH VALUE ON THE RETARDING ACTION OF FORMALDEHYDE ON FERMENTATION

Time in days	Formaldehyde, grams per 100 cc at pH 2.5			Formaldehyde, grams per 100 cc at pH 3.5		Formaldehyde, grams per 100 cc at pH 7.0	
	0	0.0037	0.0075	0.0037	0.0075	0.0075	0.015
	Loss in weight in grams per 100 cc						
1	1.20	1.66	0.15	2.34	1.14	1.13	0.60
3	4.47	5.36	0.40	6.20	2.32	3.93	3.72
6	6.53	7.94	3.78	8.21	6.96	6.57	4.46
10	7.16	9.24	6.95	8.75	7.83	8.10	5.33
15	7.44	10.11	7.56	9.13	8.44	8.72	5.77
20	7.61	10.73	7.84	9.43	8.90	9.20	6.08
24	7.78	11.23	8.05	9.67	9.26	9.58	6.34
27	8.03	11.60	8.42	10.22	.....	10.14	6.74
31	8.21	11.85	8.67	10.34	.....	10.56	7.03

Although the results are not so definite as those obtained with sodium chloride, they indicate that the preservative is somewhat more toxic at pH 2.5 and 3.5 than at 7.0. The difference is, however, far less pronounced than for sodium benzoate, salicylate, acetic acid, and sulfurous acid.

SUMMARY

The concentrations of sodium benzoate, sodium salicylate, potassium acetate ('acetic acid'), and sodium sulfite ('sulfurous acid') required to prevent the growth of yeasts, molds, and bacteria were much greater at pH 5 to 9.0 than at pH values in the distinctly acid range 2.0 to 4.5.

The effect of these preservatives on the rate of fermentation was modified in like manner by the pH value, but to a lesser degree.

The retarding effect of sodium benzoate on yeast multiplication was less at neutrality than at pH 3.0 and 3.9.

The concentrations of sodium chloride and of formaldehyde to prevent growth and the retarding action of these antiseptics on the rate of fermentation by *Saccharomyces ellipsoideus* were affected only moderately by the pH values used in these experiments.

The concentration of sodium benzoate required to preserve several food products was found to depend upon the pH value. In some instances, more than 200 times as much preservative was required at neutrality as at pH 3.0 or less.

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